Statement

I, Takashi SHOJI, of c/o YuNeed International Patent Office, 6th Floor, SN Iwamotocho Bldg., 2-10, Iwamotocho 3-chome, Chiyoda-ku, Tokyo 101-0032 Japan, do hereby declare that I am conversant with the English and Japanese languages and am a competent translator thereof. I further declare that to the best of my knowledge and belief the attached English translation of the Japanese Application No. JP2002-382083 filed on November 24, 2002 is a true and correct translation made by me.

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[Documents] Specification

[Title of the Invention] An inhibitory agent for metastasis of colon carcinoma

[Scope of Claims]

[Claim 1] An agent for inhibiting metastasis of colorectal cancer, wherein the agent inhibits the function of Asef (APC-stimulated guanine nucleotide exchange factor) and/or inhibits the expression of the Asef gene.

[Claim 2] An agent for inhibiting metastasis of colorectal cancer, wherein the agent inhibits the expression of the Asef (APC-stimulated guanine nucleotide exchange factor) gene.

[Claim 3] An agent for inhibiting metastasis of colorectal cancer, wherein the agent inhibits the binding of Asef (APC-stimulated guanine nucleotide exchange factor) to the gene product of APC (Adenomatous Polyposis Coli).

[Claim 4] An agent for inhibiting metastasis of colorectal cancer, wherein the agent inhibits the guanine nucleotide exchange factor activity of Asef (APC-stimulated guanine nucleotide exchange factor).

[Claim 5] A method for inhibiting metastasis of colorectal cancer, wherein the method comprises inhibiting the function of Asef (APC-stimulated guanine nucleotide exchange factor) and/or inhibits the expression of the Asef gene.

[Claim 6] A method for inhibiting metastasis of colorectal cancer, wherein the method comprises inhibiting the expression of the Asef (APC-stimulated guanine nucleotide exchange factor) gene.

[Claim 7] A method for inhibiting metastasis of colorectal cancer, wherein the method comprises inhibiting the binding of Asef (APC-stimulated guanine nucleotide exchange factor) to the gene product of APC (Adenomatous Polyposis Coli).

[Claim 8] A method for inhibiting metastasis of colorectal cancer, wherein the method comprises inhibiting the guanine nucleotide exchange factor activity of Asef (APC-stimulated guanine nucleotide exchange factor).

[Claim 9] An agent for inhibiting Asef, wherein the agent utilizes RNA interference to inhibit the expression of the Asef (APC-stimulated guanine nucleotide exchange factor) gene.

[Claim 10] An agent for inhibiting Asef, comprising an oligonucleotide that exhibits an RNA interference effect on the expression of the Asef (APC-stimulated guanine nucleotide exchange factor) gene.

[Claim 11] An oligonucleotide having the nucleotide sequence set forth in SEQ ID NO: 1 in the sequence listing.

[Claim 12] An oligonucleotide having the nucleotide sequence set forth in SEQ ID NO: 2 in the sequence listing.

[Claim 13] An oligonucleotide having the nucleotide sequence set forth in SEQ ID NO: 3 in the sequence listing.

[Claim 14] An oligonucleotide having the nucleotide sequence set forth in SEQ ID NO: 4 in the sequence listing.

[Claim 15] The agent according to claim 10, comprising an oligonucleotide having the nucleotide sequence set forth in SEQ ID NO: 1 or 3 in the sequence listing.

[Claim 16] A method for inhibiting Asef, wherein the method utilizes RNA interference on the expression of the Asef (APC-stimulated guanine nucleotide exchange factor) gene.

[Claim 17] A method for inhibiting Asef, wherein the method comprises utilizing an oligonucleotide exhibiting an RNA interference effect on the expression of the Asef (APC-stimulated guanine nucleotide exchange factor) gene.

[Claim 18] The method for inhibiting Asef according to claim 17, wherein the method comprises utilizing an oligonucleotide having the nucleotide sequence set forth in SEQ ID NO: 1 or 3 in the sequence listing.

[Claim 19] An agent for inhibiting metastasis of colorectal cancer, comprising the agent according to any one of claims 9, 10 and 15.

[Claim 20] An agent for inhibiting metastasis of colorectal cancer, comprising an oligonucleotide having the nucleotide sequence set forth in any one of SEQ ID NOS: 1 to 4 in the sequence listing.

[Claim 21] A method for inhibiting metastasis of colorectal cancer, wherein the method uses the agent according to any one of claims 9, 10 and 15.

[Claim 22] A method for inhibiting metastasis of colorectal cancer, wherein the method uses an oligonucleotide having the nucleotide sequence set forth in any one of SEQ ID NOS: 1 to 4 in the sequence listing.

[Claim 23] A pharmaceutical composition, comprising the agent according to any one of claims 1 to 4, 19 and 20, or the agent according to any one of claims 9, 10 and 15.

[Claim 24] An agent for preventing and/or treating colorectal cancer, comprising the agent according to any one of claims 1 to 4, 19 and 20, or the agent according to any one of claims 9, 10 and 15.

[Claim 25] A method for preventing and/or treating colorectal cancer, wherein the method uses the agent according to any one of claims 1 to 4, 19 and 20, or the agent according to any one of claims 9, 10 and 15.

[Detailed Description of the Invention]

[0001]

[TECHNICAL FIELD]

The present invention relates to a method for inhibiting metastasis of colorectal cancer and an agent for inhibiting metastasis of colorectal cancer, which are characterized by inhibiting the function of Asef (APC-stimulated guanine nucleotide exchange factor) and/or inhibiting the expression of Asef. More specifically, the present invention relates to an agent for inhibiting metastasis of colorectal cancer, an agent for inhibiting Asef, a pharmaceutical composition, an agent for preventing and/or treating colorectal cancer, a method for inhibiting metastasis of colorectal cancer, and a method for preventing and/or treating colorectal cancer, which are characterized by inhibiting the expression of Asef, inhibiting the binding of Asef to the gene product of APC (Adenomatous Polyposis Coli), or inhibiting the guanine nucleotide exchange factor (hereunder, referred to in abbreviated form as "GEF") activity of Asef.

[0002]

[BACKGROUND ART]

Asef is a protein that was found by the present inventors as a colorectal tumor suppressor gene-associated protein M1, which has already been disclosed and for which a patent application has been filed (Patent Reference 1) (Non-patent Reference 1). The protein consists of 619 amino acid residues, and contains the Dbl homology (DH) domain, the pleckstrin homology (PH) domain and the Src homology 3 (SH3) domain in its amino acid sequence.

[0003]

In terms of function, it is known that Asef has GEF activity specific for Rac that is a member of a Rho family. More specifically, Asef binds to Rac to stimulate a GDP/GTP exchange reaction which results in the activation of Rac, thereby acting on NFκB, c-jun, SRE and the like, which are located downstream of the Rac related-intracellular signal transduction.

Rho family proteins are small G proteins and play key roles in the reorganization of the actin network, thereby regulating cell migration and cell-cell adhesion. Therefore, there is a possibility that Asef induces cellular lamellipodia (lobopodium) or cell membrane ruffling and participates in cell migration and cell-cell adhesion.

[0004]

It has been revealed that the binding of Asef to the gene product of the tumor suppressor gene APC via the armadillo repeat domain of the gene product. The GEF activity of Asef is positively regulated by the APC gene product. Actually, the induction of Asef-mediated cell membrane ruffling or lamellipodia formation by the APC gene product is observed in MDCK cells that are canine kidney-derived epithelial-like cells. Further, intracellular distribution of Asef revealed that Asef accumulates at the tips of microtubules in motile cells similarly to the APC gene product when the cells migrate from the crypt to the villus tip of the colon. Therefore, Asef may hold the key in controlling cell migration in intestinal villi.

[0005]

Meanwhile, the tumor suppressor gene APC (Non-patent Reference 2) has been isolated as a responsible gene for familial adenomatous polyposis (FAP). Mutation of the gene is observed in approximately 70% to 80% of sporadic colorectal cancers. The APC gene product (hereunder, referred to as "APC") is a giant protein of approximately 300 kDa that comprises 2,843 amino acid residues. APC contains an armadillo repeat domain in the amino acid sequence thereof that participates in protein-protein interaction. Most somatic APC mutations observed in colorectal tumor cells occur within its central region called the "mutation cluster region (MCR) " and result in the generation of truncated APCs that lack the binding sites for microtubules, EB1 or hDLG, and at least some of the sites for β-catenin and Axin (Non-patent References 4, 5, 6, 7

and 8). However, the region of APC responsible for binding to Asef, the armadillo repeat domain, is retained in most mutant APCs (Non-patent References 6, 7 and 8). APC has a function to bind to β-catenin, one kind of oncogene product, to induce its degradation (Non-patent References 2, 3, 4, 5 and 6). β-catenin, which is a Wnt/Wingless signal transduction factor, binds to the cytoplasmic domain of cadherin and plays a role in cell adhesion, while it plays important roles in developmental processes and in tumorigenesis (Non-patent References 9 and 10).

[0006]

The amino acid sequence of Asef and the nucleotide sequence of its gene have been deposited with GenBank under the accession number AB042199. Further, the amino acid sequence of APC and the nucleotide sequence of its gene have been deposited with GenBank under the accession number NM000038.

[0007]

Patent Reference 1: Japanese Patent Laid-Open No.2001-057888.

Non-patent Reference 1: Kawasaki, Y., et al., Science, 2000, Vol. 289, p.1194-1197.

Non-patent Reference 2: Kinzler, K. W., et al., Cell, 1996, Vol. 87, p.159-170.

Non-patent Reference 3: Fearnhead, et al., Human Molecular Genetics, 2001, Vol. 10, p.721-733.

Non-patent Reference 4: Bienz, M., et al., Cell, 2000, Vol. 103, p.311-320.

Non-patent Reference 5: Perifer, M., et al., Science, 2000, Vol. 287, p.1606-1609.

Non-patent Reference 6: Akiyama, T., Cytokine and Growth Factor Reviews, 2000, Vol. 11, p.273-282.

Non-patent Reference 7: Miyoshi, Y., et al., Human Molecular Genetics, 1992, Vol. 1, p.229-233.

Non-patent Reference 8: Nagawa, H., et al., Human Mutation, 1993, Vol. 2, p.425-434.

Non-patent Reference 9: Cell, 1996, Vol. 86, p.391-399.

Non-patent Reference 10: Nature, 1996, Vol. 382, p.638-642.

[0008]

[Object to be Solved in the Invention]

It is known that Asef binds to the gene product of the tumor suppressor gene APC which plays important roles in tumorigenesis and in developmental processes as described in the foregoing. However, the function of Asef in cells and the relation of Asef with diseases have not yet been clarified. An object to be solved is to clarify the function of Asef and regulate the function thereof, thereby to provide a means that allows for preventing and treating diseases attributable to Asef.

[0009]

[Means for Solution]

The present inventors hypothesized based on the GEF activity of Asef and its intracellular localization that Asef may participate in cell migration and cell-cell adhesion, and found that Asef promotes the motility of colorectal tumor cells in colorectal cancers, particularly in colorectal cancers in which APC mutations are observed, and participates in the metastasis. By utilizing this finding, the present inventors found that metastasis of colorectal cancer is inhibited by inhibiting the function of Asef and/or inhibiting the expression of the Asef gene, and thereby complete the present invention.

[0010]

Thus, the present invention comprises the followings:

(1) An agent for inhibiting metastasis of colorectal cancer, wherein the agent inhibits the function of Asef and/or inhibits the expression of the Asef gene,

(2) An agent for inhibiting metastasis of colorectal cancer, wherein the agent inhibits the

expression of the Asef gene,

(3) An agent for inhibiting metastasis of colorectal cancer, wherein the agent inhibits the binding of Asef to the gene product of APC,

(4) An agent for inhibiting metastasis of colorectal cancer, wherein the agent inhibits the guanine nucleotide exchange factor activity of Asef,

(5) A method for inhibiting metastasis of colorectal cancer, wherein the method comprises inhibiting the function of Asef and/or inhibits the expression of the Asef gene,

(6) A method for inhibiting metastasis of colorectal cancer, wherein the method comprises inhibiting the expression of the Asef gene,

(7) A method for inhibiting metastasis of colorectal cancer, wherein the method comprises inhibiting the binding of Asef to the gene product of APC,

(8) A method for inhibiting metastasis of colorectal cancer, wherein the method comprises inhibiting the guanine nucleotide exchange factor activity of Asef,

(9) An agent for inhibiting Asef, wherein the agent utilizes RNA interference to inhibit the expression of the Asef gene,

(10) An agent for inhibiting Asef, comprising an oligonucleotide that exhibits an RNA interference effect on the expression of the Asef gene,

(11) An oligonucleotide having the nucleotide sequence set forth in SEQ ID NO: 1 in the sequence listing,

(12) An oligonucleotide having the nucleotide sequence set forth in SEQ ID NO: 2 in the sequence listing,

(13) An oligonucleotide having the nucleotide sequence set forth in SEQ ID NO: 3 in the sequence listing,

(14) An oligonucleotide having the nucleotide sequence set forth in SEQ ID NO: 4 in the sequence listing,

(15) The agent described in the aforementioned (10), comprising an oligonucleotide having the nucleotide sequence set forth in SEQ ID NO: 1 or 3 in the sequence listing,

(16) A method for inhibiting Asef, wherein the method utilizes RNA interference on the expression of the Asef gene,

(17) A method for inhibiting Asef, wherein the method comprises utilizing an oligonucleotide exhibiting an RNA interference effect on the expression of the Asef gene,

(18) The method for inhibiting Asef described in the aforementioned (17), wherein the method comprises utilizing an oligonucleotide having the nucleotide sequence set forth in SEQ ID NO: 1 or 3 in the sequence listing,

(19) An agent for inhibiting metastasis of colorectal cancer, comprising the agent described in any one of the aforementioned (9), (10) and (15),

(20) An agent for inhibiting metastasis of colorectal cancer, comprising an oligonucleotide having the nucleotide sequence set forth in any one of SEQ ID NOS: 1 to 4 in the sequence listing,

(21) A method for inhibiting metastasis of colorectal cancer, wherein the method uses the agent described in any one of the aforementioned (9), (10) and (15),

(22) A method for inhibiting metastasis of colorectal cancer, wherein the method uses an oligonucleotide having the nucleotide sequence set forth in any one of SEQ ID NOS: 1 to 4 in the sequence listing,

(23) A pharmaceutical composition, comprising the agent described in any one of the aforementioned (1) to (4), (19) and (20), or the agent described in any one of the aforementioned

(9), (10) and (15),

(24) An agent for preventing and/or treating colorectal cancer, comprising the agent described in any one of the aforementioned (1) to (4), (19) and (20), or the agent described in any one of the aforementioned (9), (10) and (15),

(25) A method for preventing and/or treating colorectal cancer, wherein the method uses the agent described in any one of the aforementioned (1) to (4), (19) and (20), or the agent described in any one of the aforementioned (9), (10) and (15).

[0011]

[DETAILED DESCRIPTION OF THE INVENTION]

In the present invention, it was found that Asef decreases cell-cell adhesion of epithelium-derived cells and also noticeably promotes the motility thereof. Further, it was found that these functions are regulated by APC, and particularly, it was found that truncated APC mutants that are identified in the majority of colorectal tumor cells activate Asef constitutively. Therefore, it is believed that formation of a complex between mutated APC and Asef contributes to aberrant motility of colorectal tumor cells. That is, it is concluded that the complex may be involved in the upward migration of intestinal epithelial cells, more specifically, in the migration from the crypt to the villus tip. Indeed, it has been reported that forced expression of the APC gene induces aberrant cell migration in the intestinal epithelium (Wong, M. H., et al., Proceeding of national academy of science USA」、1996, Vol. 93, p.9588-9593). It has been reported that early adenoma cells in APC knockout mice exhibit a proliferation rate similar to that of normal crypt epithelial cells, but lack directed migration along the crypt-villus axis (Oshima, H., et al., Cancer Research, 1997, Vol. 57, p.1644-1649). Aberrant migratory behavior due to Asef activation by truncated APC mutants may be thus significant for both adenoma formation and

tumor progression to invasive malignancy. In addition, Asef mutants that lack the GEF domain do not affect cell-cell adhesion and cell motility, resulting in the conclusion that GEF activity is important for such a function of Asef.

[0012]

These findings revealed that the motility of colorectal tumor cells expressing mutant APCs can be inhibited by using a dominant-negative mutant that inhibits the binding of Asef to mutant APCs, for example, a mutant consisting of the APC-binding region (amino acid sequence from the 73rd to the 126th amino acid residue) in the amino acid sequence of Asef or a mutant that lacks the GEF domain of Asef. Also revealed was that the motility of colorectal tumor cells expressing mutant APCs can similarly be inhibited by inhibiting the expression of the Asef gene or the APC gene. Further, it was found that the tumorigenicity, proliferative growth and, moreover, metastasis of human colorectal tumor cells in SCID mice can be inhibited by using the aforementioned dominant-negative mutants, or by inhibiting the expression of Asef gene or APC gene. Thus, inhibition of the function of Asef and/or inhibition of the expression of Asef gene allow for inhibiting the motility of cells and, further, inhibiting metastasis of tumor cells.

[0013]

Based on the above-described findings, the present invention provides an agent for inhibiting metastasis of colorectal cancer and a method for inhibiting metastasis of colorectal cancer, which are characterized by inhibiting the function of Asef. The agent for inhibiting metastasis of colorectal cancer and the method for inhibiting metastasis of colorectal cancer are characterized by inhibiting the function of Asef and/or inhibiting the expression of the Asef gene. Inhibition of the function of Asef can be carried out, for example, by inhibiting the binding of Asef to APC, or inhibiting the GEF activity of Asef. The binding of Asef to APC, which is the

target of inhibition, is preferably the binding of Asef to normal APC, more preferably the binding of Asef to an APC mutant, further preferably the binding of Asef to a truncated APC mutant, and still more preferably the binding of Asef to a truncated APC mutant that contains an armadillo repeat domain. Examples of a truncated APC mutant that contains an armadillo repeat domain include a polypeptide consisting of the consecutive amino acid residues from the 1st (the N terminus) to the 876th residue of the amino acid sequence of APC, or a polypeptide consisting of the consecutive amino acid residues from the 1st (the N terminus) to the 1309th residue of the amino acid sequence of APC. These polypeptides were identified as truncated APC mutants in most colorectal cancers and familial adenomatous polyposis (FAP).

[0014]

Inhibition of the expression of the Asef gene can be carried out, for example, by applying an RNA interference effect on the expression of the Asef gene. RNA interference is a method for inhibiting the expression of a gene by using RNA, as has been reported in recent years (Paddison, P. J., et al., Genes and Development, 2002, Vol. 16, p.948-958). More specifically, the expression of the Asef gene can be inhibited by using an oligonucleotide that exhibits an RNA interference effect on the expression of the Asef gene. Examples of the oligonucleotide can include a cDNA having the nucleotide sequence set forth in SEQ ID NO: 1 in the sequence listing. The complementary RNA (SEQ ID NO: 3 in the sequence listing) of the cDNA can also be used. Inhibition of the expression of the Asef gene can be carried out by transfecting a cell with a vector containing the cDNA or with the complementary RNA thereof. Transfection of a cell with the vector or with the RNA can be conducted utilizing a known method such as lipofection. Accordingly, an agent for inhibiting Asef comprising the aforementioned oligonucleotide is also included in the scope of the present invention. The agent for inhibiting

Asef may contain one kind of oligonucleotide, or may contain two or more kinds of oligonucleotide. Further, inhibition of the Asef gene expression may also be carried out by using an antisense oligonucleotide against the Asef gene. The aforementioned oligonucleotide exhibiting an RNA interference effect or the aforementioned antisense oligonucleotide can be obtained from oligonucleotides that are designed on the basis of the nucleotide sequence of the Asef gene, by selecting oligonucleotides that specifically inhibit the expression of Asef using an Asef gene expression system.

[0015]

Inhibition of the binding of Asef to APC can be carried out using a dominant-negative Asef mutant for the binding. For example, an Asef mutant that can bind to APC but does not exhibit GEF activity can be used as an agent for inhibiting the binding of Asef to APC. Such an Asef mutant can be obtained by designing mutants based on the amino acid sequence of Asef and examining their binding activity to APC according to a conventional method. More specifically, a mutant that lacks the GEF domain of Asef can be exemplified. Alternatively, a polypeptide consisting of the APC-binding region (amino acid sequence from the 73rd to the 126th amino acid residue) in the amino acid sequence of Asef is preferably used. A polypeptide that inhibits the binding of Asef to APC that is selected from polypeptides that are designed based on the amino acid sequence of this polypeptide, can also be used. Further, inhibition of the binding of Asef to APC can also be carried out by inhibiting the expression of the APC gene. Inhibition of APC gene expression can be conducted by using an oligonucleotide that exhibits an RNA interference effect on the expression of the APC gene. Examples of the oligonucleotide can include a cDNA having the nucleotide sequence set forth in SEQ ID NO: 2 in the sequence listing. Further, the complementary RNA (SEQ ID NO: 4 in the sequence listing) of the cDNA

can also be used. Alternatively, inhibition of the APC gene expression can be carried out by using an antisense oligonucleotide against the APC gene. The aforementioned oligonucleotide exhibiting an RNA interference effect or the aforementioned antisense oligonucleotide can be obtained from oligonucleotides that are designed on the basis of the nucleotide sequence of the APC gene, by selecting oligonucleotides that specifically inhibit the expression of APC using an APC gene expression system.

[0016]

Inhibition of the GEF activity of Asef can be carried out, for example, by using an inhibitor of GEF activity that can be identified using Asef. Further, a compound that inhibits the expression of the Asef gene or a compound that inhibits the binding of Asef to APC may be identified using the Asef gene or using Asef and APC, and the thus-identified compound may be used. An assay system for identifying the compound can be constructed utilizing a known screening system.

[0017]

Metastasis of colorectal cancer can be inhibited by using the aforementioned agent for inhibiting Asef. That is, an agent for inhibiting metastasis of colorectal cancer comprising an agent for inhibiting Asef and a method for inhibiting metastasis of colorectal cancer comprising using the aforementioned agent for inhibiting Asef are also included in the scope of the present invention. More specifically, an agent for inhibiting metastasis of colorectal cancer comprising an oligonucleotide having any one of the nucleotide sequences set forth in SEQ ID NOS: 1 to 4 in the sequence listing and a method for inhibiting metastasis of colorectal cancer comprising using any one of these oligonucleotides can be exemplified.

[0018]

Tumorigenicity and metastasis of colorectal cancer can be inhibited by applying the agent for inhibiting metastasis of colorectal cancer or the agent for inhibiting Asef of the present invention. More specifically, the above described agent for inhibiting metastasis of colorectal cancer or the agent for inhibiting Asef can be used in the prevention and/or treatment of colorectal cancer and colorectal cancer metastasis. From this viewpoint, an agent for preventing and/or treating colorectal cancer comprising an effective amount of the aforementioned agent for inhibiting metastasis of colorectal cancer or the agent for inhibiting Asef are also included in the scope of the present invention. Further, a method for preventing and/or treating colorectal cancer comprising using the aforementioned agent for inhibiting metastasis of colorectal cancer or the agent for inhibiting Asef can be also provided.

[0019]

A pharmaceutical composition that includes the aforementioned agent for inhibiting metastasis of colorectal cancer or the agent for inhibiting Asef can be thus provided according to the present invention. In terms of formulation of an agent for inhibiting metastasis of colorectal cancer, a pharmaceutical composition, an agent for preventing colorectal cancer, and an agent for treating colorectal cancer, it is preferable to prepare it in combination with a suitable pharmaceutical carrier. Such a formulation contains the effective amount of the agent for inhibiting metastasis of cololectal cancer, pharmaceutical composition, agent for preventing colorectal cancer, and agent for treating colorectal cancer that are provided in the present invention, as well as a pharmaceutically acceptable carrier or an excipients. The carrier can be exemplified by physiological saline, buffered physiological saline, dextrose, water, glycerol, ethanol, and mixtures thereof, but is not limited to these examples. Suitable formulation may be selected in accordance with an administration route. The formulation is well known in the art.

The agent for inhibiting metastasis of colorectal cancer, pharmaceutical composition, agent for preventing colorectal cancer, and agent for treating colorectal cancer may be used alone or may be used together with other compounds or medicaments necessary for treatment.

[0020]

In terms of an administration form of the agent for inhibiting metastasis of colorectal cancer, pharmaceutical composition, agent for preventing colorectal cancer, and agent for treating colorectal cancer that are provided in the present invention, it may be either systemic administration or local administration. One preferred mode of systemic administration is injection, for example, intravenous injection. Other injection routes such as subcutaneous injection, intraarterial injection, or intraperitoneal injection can be also employed. Other modes of administration can be oral administration, if enteric formulation or capsule formulation may be available. In addition, per mucosal administration or per cutaneous administration using a permeating agent such as bile salt, fusidic acid, or other surfactants can also be used. Topical administration can be in the forms of plaster, paste, gel and similar form.

[0021]

When using the oligonucleotide as described above, it is possible to produce the oligonucleotide into the cell of the target by use of gene therapy. The gene therapy can be performed by using a known method. For example, it can be achieved by preparing and using a replication-defective retrovirus vector containing the aforementioned oligonucleotide.

[0022]

Suitable dosage ranges depend on the following: effectiveness of the aforementioned agent for inhibiting metastasis of colorectal cancer, pharmaceutical composition, agent for preventing colorectal cancer, and agent for treating colorectal cancer; the administration route;

characteristics of the prescription; the characteristics of the conditions of the subject; and the assessment by the physician in charge. More specifically, a suitable dose may fall, for example, within a range of 0.1 μg to 100 μg per 1 kg of the body weight of the subject. However, a dosage may be altered using conventional experiments for optimization of a dosage that are well known in the art.

[0023]

For formulations, formulation means that are well-known should be introduced in accordance with the physical properties of each subject such as a peptide, protein, oligonucleotide, or compound. More specifically, a formulation method for, for example, powdered drug, pills, tablets, capsule formulations, aqueous solution formulations, ethanol solution formulations, liposome formulations, fat emulsions, or clathrates such as cyclodextrin can be employed.

[0024]

Powders, pills, capsules, and tablets can be prepared using an excipient such as lactose, glucose, sucrose, or mannitol; a disintegrant agent such as starch or sodium alginate; a lubricant such as magnesium stearate or talc; a binder such as polyvinyl alcohol, hydroxypropyl cellulose, or gelatin; a surfactant such as fatty acid ester; a plasticizer such as glycerin, and the like. For preparation of a tablet or a capsule, a pharmaceutical carrier in a solid state is used.

[0025]

A suspension can be prepared using water; sugars such as sucrose, sorbitol, or fructose; glycols such as PEG; and oils.

[0026]

Injectable solutions can be prepared using a carrier comprising a salt solution, a glucose

solution or a mixture of salt water and a glucose solution.

[0027]

Inclusion into a liposome formulation can be conducted in the following manner: by dissolving the substance of interest in a solvent (e.g., ethanol) to make a solution, adding a solution of phospholipids dissolved in an organic solvent (e.g., chloroform), removing the solvent by evaporation and adding a phosphate buffer thereto, agitating the solution and then subjecting it to sonication followed by centrifugation to obtain a supernatant, and finally, filtrating the supernatant to recover liposomes.

[0028]

A fat emulsion can be prepared in the following manner: by mixing the substance of interest, an oil ingredient (vegetable oil such as soybean oil, sesame oil, olive oil, or MCT), an emulsifier (such as a phospholipid), and the like; heating the mixture to make a solution; adding water of a required quantity; and then emulsifying or homogenizing by use of an emulsifier (a homogenizer, e.g., a high pressure jet type, an ultrasonic type, or the like). The fat emulsion may be also lyophilized. For conducting lipid-emulsification, an auxiliary emulsifier may be added, and examples thereof include glycerin or saccharides (e.g., glucose, sorbitol, fructose, etc.).

[0029]

Inclusion into a cyclodextrin formulation may be carried out in the following manner: by dissolving the substance of interest in a solvent (e.g., ethanol); adding a solution of cyclodextrin dissolved in water under heating thereto; chilling the solution and filtering the precipitates; and drying under sterilization. At this time, the cyclodextrin to be used may be appropriately selected from among those having different void sizes (α, β, or γ type) in accordance with the bulkiness of the substance of interest.

[0030]

[EXAMPLES]

Hereinafter, the present invention may be explained more particularly with examples; however, the present invention is not limited to the following examples.

First, the following definitions relate to Asef, APC, and the mutants thereof that are used in the examples herein. The proteins and the mutants are referred to in abbreviated form.

Asef-full is a protein consisting of the wild-type, full-length Asef. It was expressed as a haemagglutinin (HA)-tagged fusion protein (HA-tagged wild-type Asef) or a Glutathione S-transferase (GST) fusion protein (GST-Asef-full).

Asef-ΔAPC is a mutant that lacks the N-terminal APC-binding region of Asef. This mutant possesses stronger GEF activity than wild-type Asef.

Asef-ΔDH is a mutant that lacks the DH domain (GEF domain) of Asef. This mutant does not exhibit GEF activity.

Asef-ABR is a polypeptide consisting of the APC-binding region (amino acid sequence from the 73rd to the 126th residue) in the amino acid sequence of Asef. It was expressed as a maltose-binding protein (MBP) fusion protein (MBP-Asef-ABR).

APC-arm is a polypeptide consisting of the armadillo repeat domain of APC. It was expressed as a Myc-tagged fusion protein (Myc-tagged APC-arm).

APC-876 is a polypeptide consisting of the consecutive amino acid residues from the 1st (the N terminus) to the 876th residue of the amino acid sequence of APC, which contains the armadillo repeat domain.

APC-1309 is a polypeptide consisting of the consecutive amino acid residues from the 1st (the N terminus) to the 1309th residue of the amino acid sequence of APC, which contains the

armadillo repeat domain.

APC-876 and APC-1309 are truncated APC mutants that were identified in colorectal tumors and familial adenomatous polyposis (FAP).

[0031]

Adenoviruses that contain DNA encoding any of them were prepared by cloning polynucleotides that encode each protein into the pAdeno-X adenoviral vector using the Adeno-XTM Expression System (Clontech).

[0032]

Plasmids that contain DNA encoding any of them were prepared according to a conventional method.

[0033]

Cell culture and transfection of the aforementioned plasmids were carried out as described in the following. MDCK cells (epithelial-like cell line established from a normal canine kidney) and WiDr cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (FCS). SW480 cells were cultured in Leibovitz's L-15 medium supplemented with 10% FCS. DLD-1 cells and HCT15 cells were cultured in RPMI 1640 medium supplemented with 10% FCS. HCT116 cells were cultured in McCoy's 5A medium supplemented with 10% FCS. These cells were transfected with the aforementioned plasmids using LipofectAMINE 2000 (Life Technologies).

[0034]

Preparation and expression of proteins were carried out in the following manner. Proteins fused to GST or MBP were synthesized in Escherichia coli and isolated by absorption to glutathione Sepharose (GSH-Sepharose; Pharmacia) or amylose resin (New England Biolabs).

[0035]

Short hairpin RNAs (hereunder, referred to in abbreviated form as "shRNA"), such as shRNA-Asef and shRNA-APC used in the RNA interference experiments were designed so as to inhibit the expression of the Asef gene and the APC gene, respectively. The nucleotide sequences of shRNA-Asef and shRNA-APC are set forth in SEQ ID NO: 1 and SEQ ID NO: 2 in the sequence listing, respectively. Further, mutations were added to shRNA-Asef and shRNA-APC to prepare shRNAs that did not inhibit the expression of the Asef gene and the APC gene. These are mut-shRNA-Asef and mut-shRNA-APC, which are set forth in SEQ ID NO: 5 and SEQ ID NO: 6 in the sequence listing, respectively.

[0036]

[Example 1]

In order to examine the effects of Asef on cell-cell adhesion and cell morphology, MDCK cells were infected with the above-described adenoviruses. The adenoviruses used were AdAsef-full, AdAsef-ΔAPC, AdAsef-ΔDH and AdAPC-arm. AdLacZ was used as a control. Immunofluorescence staining showed that the infection efficiency of the adenoviruses to MDCK cells was 90% or more. Immunoblot analysis showed that each of these viruses produces a protein of the expected size when infected into MDCK cells.

[0037]

Cell morphology was examined as follows. Infected cells were plated in 12-well tissue culture plates to get 3.0×10^4 cells per well. After 3 h of incubation at 37 °C, cells were infected with adenoviruses (multiplicity of infection (MOI) = 200), cultured for a further 36 h and then observed by a phase-contrast microscope. Cells infected with AdAsef-ΔAPC became flattened onto the substratum and exhibited membrane ruffles and lamellipodia. In contrast, cells infected

with AdAsef-ΔDH showed no morphological changes and resembled uninfected cells.

[0038]

Cell-cell adhesion was examined as follows. Infected cells were scraped from plates in phosphate-buffered saline (PBS) containing 0.02% ethylenediamine tetraacetic acid (EDTA) and subjected to pipetting 20 times. The number of cell clusters (particles) was then counted. The cell-cell adhesion was evaluated from a value obtained by dividing the number of cell clusters by the total number of cells (Np/Nc). When cells were dispersed by pipetting, cells infected with AdAsef-ΔAPC dissociated efficiently, whereas uninfected cells and cells infected with AdAsef-ΔDH or AdLacZ remained as clusters (Figure 1). These results showed that Asef has a function to decrease cell-cell adhesion, and that its GEF activity is essential for this function.

[0039]

Further, immunohistochemical analysis using an anti- E-cadherin antibody showed that overexpression of either AdAsef-full or AdAsef-ΔAPC resulted in decreased amounts of E-cadherin localized at the sites of cell-cell contact and enhanced amounts of E-cadherin localized in the cytoplasm. Immunohistochemical analysis was conducted as follows. After 36 h of adenovirus infection, MDCK cells were fixed with 3.7% formaldehyde in PBS. The fixed cells were double-stained with either a rat monoclonal antibody against E-cadherin (ECCD-2; Calbiochem) and trimethylrhodamine isothiocyanate -conjugated phalloidin (TRITC-conjugated phalloidin: Molecular Probes), or the rat mAb against E-cadherin and a rabbit pAb against β-catenin (SantaCruz Biotechnology) for 60 min at room temperature. Staining patterns obtained with anti-E-cadherin antibody and anti-β-catenin antibody were visualized with fluorescein isothiocyanate-labelled anti-rat IgG antibody and TRITC-labelled anti-rabbit IgG antibody, respectively. The cells were photographed with a Carl Zeiss LSM510 laser scanning microscope.

Staining with anti-β-catenin antibody showed a decreased amount of β-catenin localized at the site of cell-cell contact, although the decreased amount was not as prominent as that of E-cadherin. In contrast, cells infected with AdAsef-ΔDH or AdLacZ did not show any change in localization of E-cadherin or β-catenin. These results suggest that GEF activity of Asef is important for the changes in the localization of these molecules. Immunoblot analysis of MDCK cell lysates showed that the total amount of E-cadherin or β-catenin did not change significantly upon infection with AdAsef-full or AdAsef-ΔAPC. These results demonstrated that the decrease in cell-cell adhesion resulting from the expression of the Asef gene is due to a decrease in E-cadherin and β-catenin at the sites of cell-cell contact.

[0040]

[Example 2]

The effects of Asef on cell motility were examined using MDCK cells that were made to express the Asef gene, the APC gene, or mutant genes of these using the plasmids described above. Cell motility was examined by cell migration assays using Transwell migration chambers. The chambers used for MDCK cells were 12 mm in diameter with a pore size of 12 μm (Costar Corporation). After 18 h of transfection, 3.0×10^4 cells of MDCK cells were added to the upper compartment of the chamber and allowed to migrate toward the underside of the upper chamber for 18 h. Cell migration was determined by measuring the cells that had migrated to the lower side of the polycarbonate filters.

[0041]

Cells infected with a plasmid that contains DNA encoding Asef-full showed enhanced motility as compared with parental cells (MDCK) or vector-transfected cells (Mock) (Figure 2). Cells that were made to co-express the Asef-full gene together with any one of the APC-arm

gene, the APC-876 gene and the APC-1309 gene were more motile than cells transfected with the Asef-full gene alone. In the effect of APC on the ability of Asef to promote cell-motility, APC-arm, APC-876 and APC-1309 were stronger than APC-full. In contrast, APC-arm alone did not promote migration. In addition, cells transfected with the Asef-ΔAPC gene showed a further enhanced migration reaction as compared with cells cotransfected with the Asef-full gene and the APC-arm gene. These results showed that Asef has the potential to promote the migration of MDCK cells. It was shown that this potential of Asef is further enhanced by APC, particularly a truncated APC mutant that contains an armadillo repeat domain (Asef-Arm). In addition, Asef-ΔDH did not promote the migration of MDCK cells, indicating that the GEF activity of Asef is required for such migration stimulatory activity.

[0042]

Meanwhile, when the Asef-ABR gene was expressed together with the APC-1309 gene, enhancement of cell migration was almost completely inhibited. Asef-ΔDH also inhibited APC-1309 mediated enhancement of cell migration. These results indicate that the APC mutants, APC-879 and APC-1309, which have been identified in colorectal cancers and FAP, interact with Asef, and enhance its activity, thereby promoting cell migration. On the other hand, when the full-length APC gene was transfected into MDCK cells, no enhancement of Asef-induced migration was observed (Figure 2). This indicates that APC may not be an efficient activator of Asef until APC is activated by truncation in colorectal tumor cells.

[0043]

Next, the motility of SW480 cells that are known to include Asef and truncated APC mutants was examined. When SW480 cells were transfected with plasmids that contain DNA encoding Asef—ABR, the migration of the cells decreased to about 50% of that of the parental

cells or Mock cells (Figure 2). Similarly, the migration of SW480 cells transfected with Asef-ΔDH plasmids decreased by about 40%. These results demonstrated that Asef-ABR or Asef-ΔDH expressed in cells acts on the binding of Asef to truncated APC mutants in a dominant-negative manner, thereby inhibiting the cell migration by Asef-ABR or Asef-ΔDH.

[0044]

[Example 3]

The binding of Asef to truncated APC mutants in colorectal tumor cells was examined as follows. First, 5.0×10^6 cells of SW480 cells were lysed in 500 μl of buffer A (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 5 mM EDTA, 2 mM sodium vanadate (Na_3VO_4) and 10 mM sodium fluoride) containing 1% Triton X-100. The lysate was incubated with 2 μg of anti-Asef antibody for 1 h at 4°C, and then the immunocomplex was adsorbed to protein G-Sepharose 6B for 2 h at 4 °C. After washing extensively with buffer A containing 1% Triton X-100, the sample was resolved by SDS-PAGE, and transferred to a polyvinylidene difluoride membrane filter (Immobilon P; Millipore). The blot was analyzed by immunoblot analysis using alkaline phosphatase-conjugated mouse anti-rabbit IgG antibody (Promega) as a second antibody. The rabbit anti-Asef polyclonal antibody used was prepared by a conventional method (Kawasaki, Y., et al., Science, 2000, Vol. 289, p.1194-1197).

[0045]

The results showed that Asef co-immunoprecipitated with the truncated APC mutant (Figure 3a). Co-immunoprecipitation of Asef and the APC mutant was inhibited by preincubation of the antibody with an excess of the antigen for 2 h at 4 °C. These results demonstrate that Asef is associated with APC mutants in SW480 cells.

[0046]

Next, co-immunoprecipitation of GST-Asef-full and APC-arm was conducted in vitro to examine the effect of Asef-ABR addition. First, APC-arm was produced by in vitro translation (IVT-APC-arm), and incubated with GST-Asef-full bound to Sepharose in the presence of MBP-Asef-ABR. The relative amounts of APC-arm to MBP-Asef-ABR were varied as indicated in Figure 3b. APC-arm bound to GST-Asef-full-Sepharose was visualized by SDS-PAGE followed by autoradiography (top panel of Figure 3b). MBP-Asef-ABR added to the reaction mixture was visualized by subjecting the gel to Coomassie blue staining (bottom panel of Figure 3b). The results showed that the amount of co-immunoprecipitate of APC-arm and GST-Asef-full decreased in a dose-dependent manner along with the increasing amounts of Asef-ABR added. More specifically, it was revealed that Asef-ABR inhibits the binding of Asef to the APC mutant in vitro.

[0047]

Thus, the inhibition of the migration of SW480 cells was achieved using Asef-ABR that inhibits the binding of Asef to the APC mutant in a dominant negative manner.

[0048]

[Example 4]

Various colorectal tumor cell lines were infected with adenoviruses that contain DNA encoding Asef-full, Asef-ΔAPC or Asef-ΔDH, and assessed by cell migration assays in the same manner as in Example 2. The colorectal tumor cell lines used were SW480, DLD-1, HCT15, WiDr and HCT116. SW480 cells, DLD-1 cells, HCT15 cells and WiDr cells contain truncated APC mutants. HCT116 cells contain normal APC but mutated β-catenin.

[0049]

The results are shown in Figure 4. When SW480 cells were infected with AdAsef-full or

AdAsef-ΔAPC, their motility was enhanced. Further, when SW480 cells, DLD-1 cells, HCT15 cells and WiDr cells were infected with AdAsef-ΔDH, their motility was partially inhibited. In contrast, the motility of HCT116 cells was not inhibited by AdAsef-ΔDH. This indicates that full-length APC in HCT116 is unable to induce the activation of Asef. These results demonstrated that the activation of Asef is induced in colorectal tumor cells that contain truncated APC mutants, while the activation of Asef is not or is hardly induced in cells that contain normal APC. The results also showed that the activation is inhibited by Asef-ΔDH.

[0050]

[Example 5]

The interaction of Asef with APC mutants in the migration of colorectal tumor cells was investigated using RNA interference (RNAi) experiments. The experiments were carried out using the pSHAG-1 vector system (Paddison, P. J., et al., Genes and Development, 2002, Vol. 16, p.948-958). The colorectal tumor cell lines used were SW480 cells, WiDr cells, LS180 cells and HCT116 cells. SW480 cells and WiDr cells contain truncated APC mutants. LS180 cells and HCT116 cells contain normal APC but mutated β-catenin.

[0051]

Cell migration assays were carried out in the same manner as in Example 2 to assess various colorectal tumor cells transfected with expression vectors that contain either shRNA-Asef or shRNA-APC that is a short hairpin RNA. The results showed that SW480 cells and WiDr cells that were transfected with either shRNA-Asef or shRNA-APC exhibited decreased motility as compared with cells that were transfected with mut-shRNA-Asef or mut-shRNA-APC (Figure 5). In contrast, this phenomenon was not observed in LS180 cells and HCT116 cells.

[0052]

Next, immunoblot analysis was carried out in the same manner as in Example 3 to assess cells transfected with expression vectors that contain oligonucleotides encoding any one of shRNA-Asef, shRNA-APC, mut-shRNA-Asef and mut-shRNA-APC. Meanwhile, changes in α-tubulin were measured as a control. The results showed that shRNA-Asef and shRNA-APC almost completely inhibited the expression of the Asef gene and the APC gene, respectively.

[0053]

It was thus demonstrated that the motility of colorectal tumor cells that contain truncated APC mutants is decreased by inhibiting the expression of the Asef gene or the APC gene. More specifically, the results indicated that interaction of Asef with APC mutants plays an important role in the migration of colorectal tumor cells.

[0054]

[Example 6]

Cells prepared by making the Asef dominant-negative mutant Asef-ABR express in human SW480 colorectal tumor cells were respectively transplanted to SCID mice to observe changes in their tumorigenicity or proliferation. Asef-ABR plasmids were transfected into SW480 colorectal tumor cells by lipofection. The thus-obtained 3 clones were respectively cultured in vitro using L-15 medium containing G418 at a final concentration of 1 mg/ml, and then transplanted subcutaneously in the flanks of 8-week-old SCID mice at 1×10^7 cells/0.1 ml/mouse (2 to 4 individuals per group). Twenty days after transplanting the tumor cells, tumor lumps were excised to measure their weights. The weight of tumor lumps (T) of each mouse that was transplanted with the clone was divided by the value of a control group (C) to get an inhibition ratio (abbreviated as "IR") that was expressed as a percentage [IR (%) = T/C×100].

The expression of Asef-ABR in the transplanted cells was confirmed by a conventional method.

[0055]

Decreased tumorigenicity or delayed proliferation was seen in 2 out of 3 clones stably expressing Asef-ABR alone (Table 1). Thus, it is believed that Asef participates in tumorigenicity or tumor cell proliferation.

[0056]

[Table 1]

group	weight ±SD (g)	IR(%)	number of mice with tumor /transplanted with tumor
SW480	0.496 ±0.080	0	4/4
ABR-2	0.609 ±0.069	−22.7	3/3
ABR-8	0.000 ±0.000	100	0/3
ABR-17	0.203 ±0.056	59.1	4/4

[0057]

[Example 7]

Asef-ABR clones (see Example 6) that were prepared using the human HT29 colorectal tumor cell line were transplanted into SCID mice to observe changes in their tumorigenicity or proliferation. 15 mg of Asef-ABR plasmids were transfected into 5×10^6 cells of HT29 cells by lipofection. The thus-obtained 5 clones were respectively cultured in vitro using DMEM medium containing G418 at a final concentration of 1 mg/ml, and then transplanted into the spleen of 8-week-old SCID mice at 1×10^6 cells/0.05 ml/mouse (4 individuals per group). Eighteen days after transplanting the tumor cells, the mice were injected with ink via tail vein, and then sacrificed with bleeding under anesthesia with ether. The spleen and the liver were excised to measure their weights.

[0058]

Tumor formation in the spleen and the liver was not observed in 4 out of 5 clones that are the stable transfectant expressing Asef-ABR dominant-negative mutant, which were prepared using HT29 cells (Table 2). The same phenomenon was also observed in 3 clones prepared in Example 6 using SW480 colorectal tumor cells. Thus, it was demonstrated that Asef participates in tumorigenicity and tumor cell proliferation. Further, no tumor formation in the liver was observed when using the stable transfectant expressing Asef-ABR dominant-negative mutant, indicating that Asef-ABR dominant-negative mutants inhibit tumor metastasis.

[0059]

[Table 2]

group	liver weight ± SD (g)	spleen weight ± SD (mg)
normal	1.340 ±0.176	30.8 ± 7.1
parental cell line (HT29)	2.236 ±0.153	124.5 ± 20.4
Asef-ABR-A7	1.584 ±0.093	38.0 ± 3.9
Asef-ABR-B5	2.627 ±0.392	129.3 ± 13.5
Asef-ABR-C3	1.579 ±0.124	44.3 ± 11.0
Asef-ABR-C12	1.526 ±0.070	37.8 ± 5.1
Asef-ABR-D11	1.359 ±0.173	37.3 ± 4.6

[0060]

[Advantage of the Invention]

In the present invention, it was found that Asef enhances the motility of cells, and that it decreases cell-cell adhesion, and that this function of Asef is activated by the gene product of the tumor suppressor gene APC. Further, it was found that Asef enhances the motility of the colorectal tumor cells and participates in the tumorigenicity and metastasis thereof in colorectal cancers, particularly in colorectal cancers in which mutant APCs are observed. According to the

present invention based on these findings, the agent for inhibiting metastasis of colorectal cancer and the method for inhibiting metastasis of colorectal cancer, which inhibit the function of Asef and/or inhibit the expression of Asef gene, were provided. These inventions have a significant effect for the prevention and/or treatment of colorectal cancer and colorectal cancer metastasis.

[0061]

[SEQUENCE LISTING FREE TEXT]

SEQ ID NO: 1: Designed oligonucleotide based on the nucleotide sequence of human Asef to inhibit the expression of the Asef gene.

SEQ ID NO: 2: Designed oligonucleotide based on the nucleotide sequence of human APC to inhibit the expression of the APC gene.

SEQ ID NO: 3: Designed oligonucleotide based on the nucleotide sequence of human Asef to inhibit the expression of the Asef gene.

SEQ ID NO: 4: Designed oligonucleotide based on the nucleotide sequence of human APC to inhibit the expression of the APC gene.

SEQ ID NO: 5: Designed oligonucleotide based on the nucleotide sequence set forth in SEQ ID NO: 1.

SEQ ID NO: 6: Designed oligonucleotide based on the nucleotide sequence set forth in SEQ ID NO: 2.

[0062]

[Sequence Listing]

SEQUENCE LISTING

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<120> An inhibitory agent for metastasis of colon carcinoma

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[BRIEF DESCRIPTION OF THE DRAWINGS]

[Figure 1] This figure illustrates decreased cell-cell adhesion of MDCK cells infected with adenoviruses that contains DNA encoding Asef. As shown in the vertical axis, cell-cell adhesion is represented by a numerical value obtained by dividing the number of cell clumps (Np) by the total number of cells (Nc). Cells were infected with adenoviruses containing a gene encoding full-length Asef (denoted by "Asef-full"), a gene encoding the armadillo repeat domain of the APC (denoted by "APC-arm"), a gene encoding an Asef mutant that lacks the APC-binding region (denoted by "Asef-ΔAPC"), or a gene encoding an Asef mutant that lacks the DH domain (denoted by "Asef-ΔDH"), as shown in the figure. The results are shown as mean ± standard deviation (SD) obtained over three independent experiments.

[Figure 2] This figure illustrates motility of MDCK cells or SW480cells in which the Asef gene, APC gene, or the mutant gene thereof were made to express. The results are shown as the relative migration as compared to that of the parental cells.

[Figure 3] Figure 3a illustrates the binding of Asef to truncated APC mutants in SW480 cells as a result of analysis carried out by immunoprecipitation using an anti-Asef antibody (Anti-Asef). In the figure, the symbol + indicates that the antibody used was pre-incubated with antigen before immunoprecipitation. Figure 3b illustrates that Asef-ABR inhibits the interaction of IVT-APC-arm with GST-Asef-full in vitro in a dose dependent manner. In the figure, "MW." represents a molecular marker.

[Figure 4] This figure illustrates motility of various colorectal tumor cells (SW480, DLD-1, HCT15, WiDr and HCT116) in which the Asef gene or the mutant gene thereof was made to express. The results are shown as the relative migration as compared to that of each cell expressing the LacZ gene as a control.

[Figure 5] This figure illustrates that the short hairpin RNAs, shRNA-Asef and shRNA-APC, which inhibit the expression of the Asef gene and the APC gene respectively, both decreased the motility of colorectal tumor cells having an APC mutation (SW480 and WiDr), while no effect was observed on the motility of colorectal tumor cells having normal APC (HCT116 and LS180). As controls for comparison, the short hairpin RNAs, mut-shRNA-Asef and mut-shRNA-APC, which do not inhibit the expression of Asef gene or APC gene, were used. The results are shown as the relative migration as compared to that of each cell transfected with mut-shRNA-Asef.

[Documents] Drawings

Figure 1

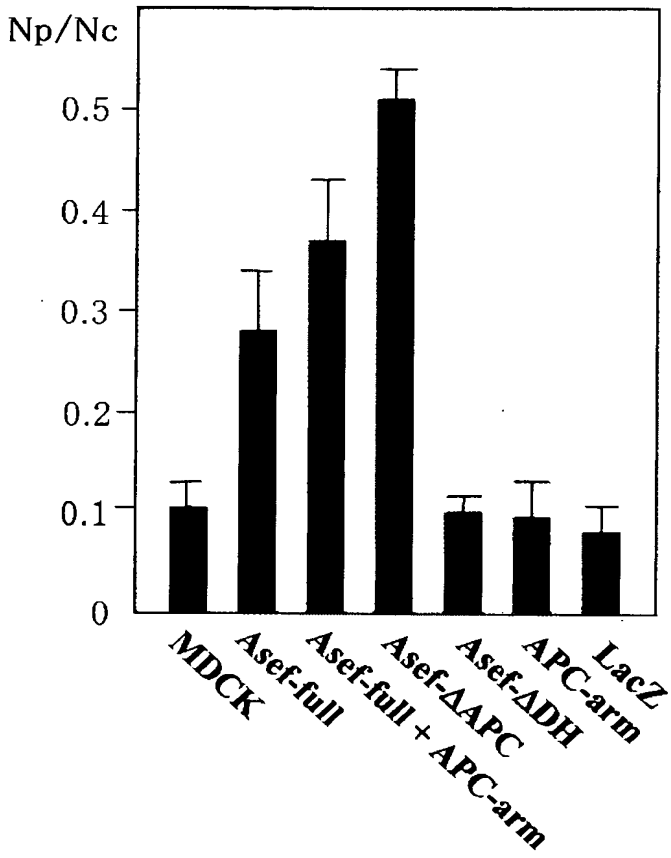
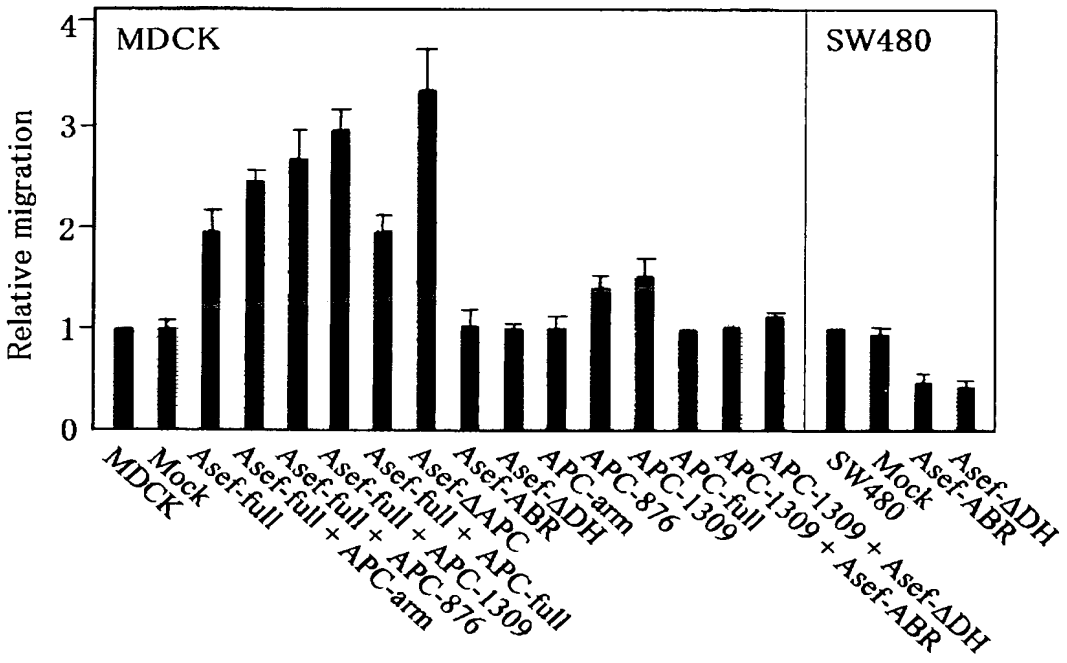


Figure 2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SW480
Relative migration
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1
2
3
4
MDCK
Mock
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Asef-full + APC-876
Asef-full + APC-1309
Asef-full + APC-full
Asef-ΔAPC
Asef-ABR
Asef-ΔDH
APC-arm
APC-876
APC-1309
APC-full
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Asef-ΔDH
APC-1309 + Asef-ΔDH

Figure 3

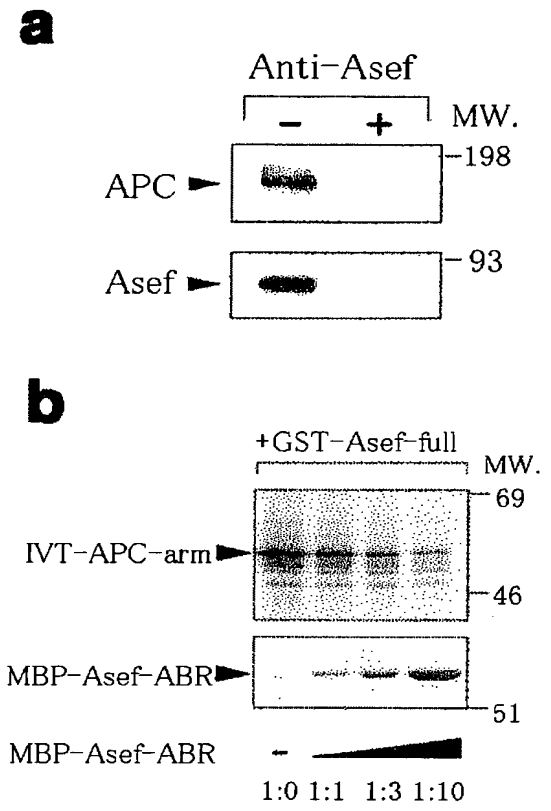
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Figure 4

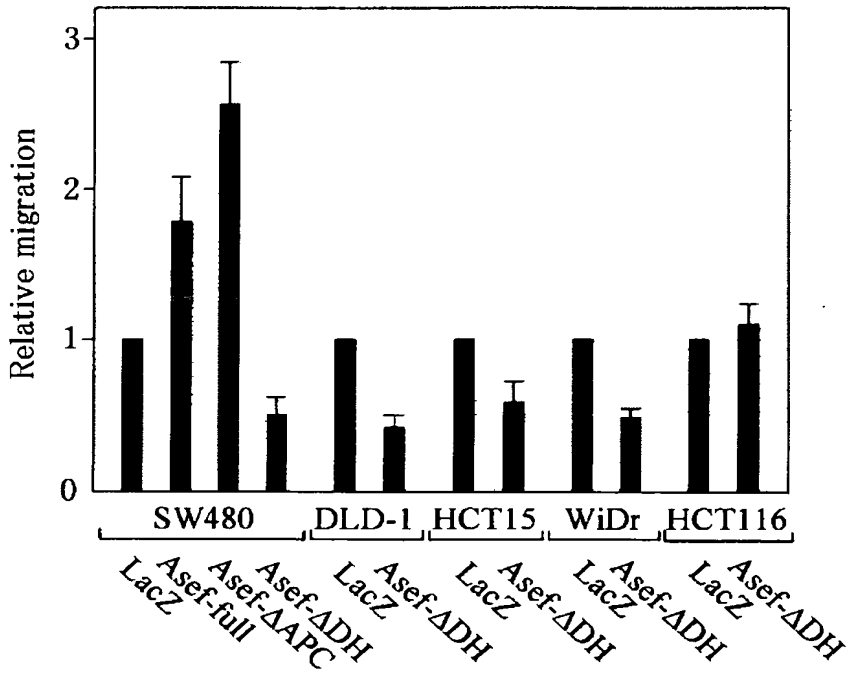
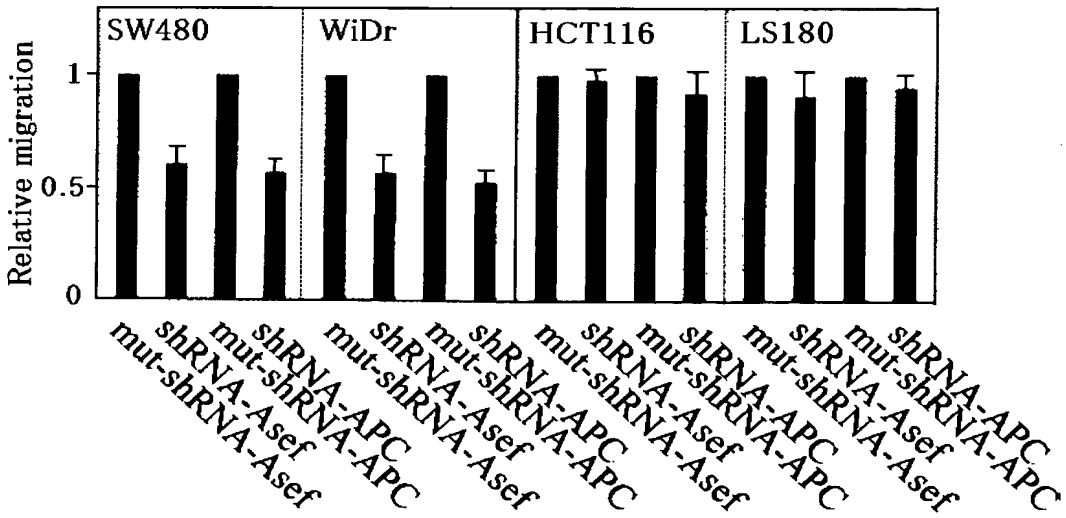


Figure 5



[Documents] Abstract

[Abstract]

[Problem] To clarify intracellular function of Asef, a protein that binds to a gene product of tumor suppressor gene APC that plays an important role in tumorigenesis and in developmental processes, and control the function, thereby to provide means that allows for preventing and treating disorders attributable to Asef.

[Means for solution] An agent for inhibiting metastasis of colorectal cancer and a method for inhibiting metastasis of colorectal cancer, which inhibit the function of Asef (i.e., binding activity to the APC gene product or guanine nucleotide exchange factor activity), and/or inhibit the expression of the Asef gene.

[Elected Drawing] none